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Optimization of conditions for bioethanol production from potato peel waste

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Abstract
Potato peel waste (PPW) is a zero value by product, which occurs in big amounts after industrial potato processing and can range from 15-40 % of initial product mass, depending on the peeling method. PPW has contained high starch content and could be utilized for bioethanol production. Four yeast isolates were used for optimization of conditions by SHF method for ethanol production from potato peel wastes at different temperatures (30, 35 and 40°C), pH (5.0, 6.0 and 7.0) and incubation period under stationary condition. Maximum bioethanol production was observed from PPW at temperature 35°C and pH 6.0 after 72 h of incubation. SSF of PPW was observed at temperature 35°C and pH 6.0 after 72 h of incubation period using YPO3 and YPmp3. Maximum ethanol 2.83% was observed by using YPO3 after 72 h of incubation, whereas 3.75% was observed using *Saccharomyces cerevisiae* after 48 h of incubation from PPW.

Keywords: Potato peel waste, Bioethanol, Yeast, Enzyme, *Saccharomyces cerevisiae*

Introduction
Worldwide, increasing research and development efforts have been directed towards the commercial production of ethanol as the most promising biofuel from renewable biomass. According to the current energy demand, environmental status, depleting of fossil fuels and hike in oil prices, there is an urgent need to search an alternative fuels which are renewable, cost effective, ecofriendly and has fewer greenhouse gases emission (Goldemberg, 2007) [6]. Bioethanol is one of the most promising alternatives to fossil fuels that can ensure energy security and address environmental pollution problems (Wang *et al*., 2016) [15]. Bioethanol is produced from agricultural residues, ligno-cellulosic and starchy biomass. The ligno-cellulosic biomass is cheaper and available in plenty but its conversion to bioethanol involves many steps and expensive (Kulkarni *et al*., 2015) [9]. The primary motive for producing fuel ethanol is to reduce the foreign exchange burden of oil imports. In this context, the starchy substrates are used for bioethanol production due to their economic viability as well as availability in large quantities in the world (Reddy *et al*., 2005) [12]. Starchy crops like corn, barley, wheat, rice, sweet sorghum and tuber crops *viz*. potatoes, sweet potatoes are being exploited for the production of ethanol since they are rich in starch, cellulose and sugars which make them cheap substrates for fuel ethanol production (Duruyurek *et al*., 2015) [5].
Starch is a complex carbohydrate which needs conversion into simple sugars before being converted into bioethanol. The production of bioethanol from starchy biomass involves three step processes: 1) liquefaction of starch by an endoamlyase such as α- amylase, 2) enzymatic saccharification of liquefied product to produce glucose; and 3) fermentation of glucose to bioethanol by yeast (Chaudhary *et al*., 2014) [3]. Different process has been used for bioethanol production from starchy biomass i.e. Separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF). SSF process is advanced, cost-effective, less time consuming and effective technology for bioethanol production using different substrates such as sweet sorghum (Yu *et al*. 2008) [16] and potato mash (Srichuwong *et al*., 2009) [13]. It combines the hydrolysis and fermentation process in a single vessel while minimizing the substrate inhibition effect and overall reaction time (Neves *et al*., 2006; Josefin, 2011) [11, 7].
Potato (*Solanum tuberosum*) is an economical food, and it provides a source of low cost energy to human diet. It has become one of the most popular crops in India and cultivated in almost all states. India ranks second next to China in global production of potato and production is about 48 million tons per annum.

About 68% of potato production is consumed as fresh while rest is utilized as seed (8.5%) and processing purposes (7.5%), while the remaining 16% goes as waste during storage and transportation. Potatoes are starchy crops, which do not require complex pre-treatments. Although, it is also a high value crop, from 5% to 20% ends as waste potato by-products from potato cultivation and could be utilized for bioethanol production (Adarsha *et al*., 2010; Minal and Deshpande, 2010) [1, 10]. Moreover, during potato processing, particularly in the potato chips industry, approximately 18% of the potatoes are generated as waste. Waste potatoes are produced as a by-product in potato cultivation. In food potato industry, a lot of solid potato mash is also formed which can be considered as a raw material for bioethanol production (Kilpimaa *et al*., 2009) [8]. The problem of the management of potato peel waste (PPW) causes considerable concern to the potato industries, thus implying the need to identify an integrated, ecofriendly solution. Potato peel is a zero value waste from potato processing plants (Duruyurek *et al*., 2015) [5]. Losses caused by potato peeling ranging from 15-40% which is depended on the processed applied steam, abrasion or lye peeling for instance (Arapoglou *et al*., 2010) [2]. Therefore, the waste from the potato industry can also be utilized as growth media (carbon source) for the fermentation processes in bioethanol production as it has high starch content. Consequently, there is an urgent need to find an alternative productive use of the potato peels for biofuel production. The PPW contains a good amount of starch, cellulose, hemi-cellulose and fermentable sugars which are sufficient for yeast growing and can serve as a feedstock for bioethanol production (Duhan *et al*., 2013) [4]. Therefore, present study was investigated for the optimization of conditions for bioethanol production from potato peel waste (PPW).

Material and methods

A. Enzymatic hydrolysis and saccharification of potato peel wastes

Potato peel waste (PPW) was hydrolysed enzymatically using crude bacterial enzyme (1.0% v/v) and incubated in water bath for 120 min at 80°C. Total reducing sugar released was measured by standard DNS method after centrifuge the samples at 10,000 rpm for 15 min.

B. Optimization of fermentation conditions for bioethanol production from potato peel waste (PPW) using selected yeast isolates

The bioethanol production from selected yeast isolates at different conditions was optimized under separate hydrolysis and fermentation (SHF) process as described below:

Effect of temperature

To determine the optimum temperature for maximum bioethanol production by selected isolates, after the hydrolysis process, each flask containing 1.0% PPW hydrolysate was inoculated with 1.0% (v/v) yeast isolates and incubated at different temperature *viz*., 30°C, 35°C and 40°C under stationary conditions. Ethanol production was estimated at different incubation period by the potassium dichromate method.

Effect of pH

To determine the optimum pH for maximum bioethanol production by selected isolates, after the hydrolysis process, each flask containing 1.0% PPW hydrloysate was inoculated with 1.0% (v/v) yeast isolates and incubated at temperature 35°C with different pH *viz*., 5.0, 6.0 and 7.0 under stationary conditions. Ethanol production was recorded at different incubation period by the potassium dichromate method.

Effect of carbon and nitrogen sources

Different carbon (@ 1.0% glucose and maltose) and nitrogen (@ 0.1% Yeast extract and peptone) sources were used to determine their effect on bioethanol production. Each flask containing 1.0% PPW hydrolysate contained either of glucose or maltose as carbon source and yeast extract or peptone as a nitrogen source was inoculated with 1.0% (v/v) yeast isolates and incubated at optimized temperature for different time interval for estimation of ethanol.

Effect of incubation period

To study the effect of incubation period, in each flask containing 1.0% PPW hydrolysate inoculated with 1.0% (v/v) yeast isolates then incubated for different period. After incubation, the samples analysed for ethanol estimation.

All the experiments were performed in triplicates and the data was analyzed by the application of complete randomized design (CRD) using OPSTAT software.

Results and discussion

Hydrolysis and saccharification of potato peel waste was done by using crude enzyme (bacterial) at 80°C for 2 h. Maximum 0.56 g/ml (PPW) reducing sugars were released after 120 min on loading of 1.0% enzyme (Fig 1).

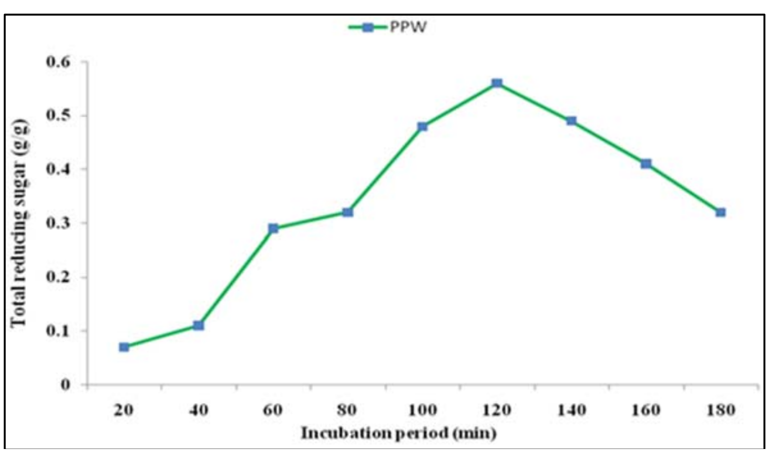

Fig 1: Total reducing sugars released from PPW during hydrolysis and saccharification

A. Optimization of conditions for ethanol production from potato peel waste (PPW)

Ethanol production from PPW was carried out in separate hydrolysis and fermentation (SHF) process using four yeast isolates (YPmp1, YPmp3, YPO1 and YPO3). The effect of different parameters such as temperature, pH, carbon and nitrogen sources and incubation period was observed at stationary conditions. The ethanol production by hydrolysed PPW @ 1.0% was studied at different temperatures (30, 35 and 40°C) using four yeast isolates. Maximum 0.27% (PPW) ethanol was produced after 72 h of fermentation at 35°C at 1.0% inoculum concentration of YPO3 (Fig 2). All these yeast isolates produced 0.09 to 0.19% ethanol from PPW at 30°C. The ethanol production was found to be negligible at 40°C in both the substrates.

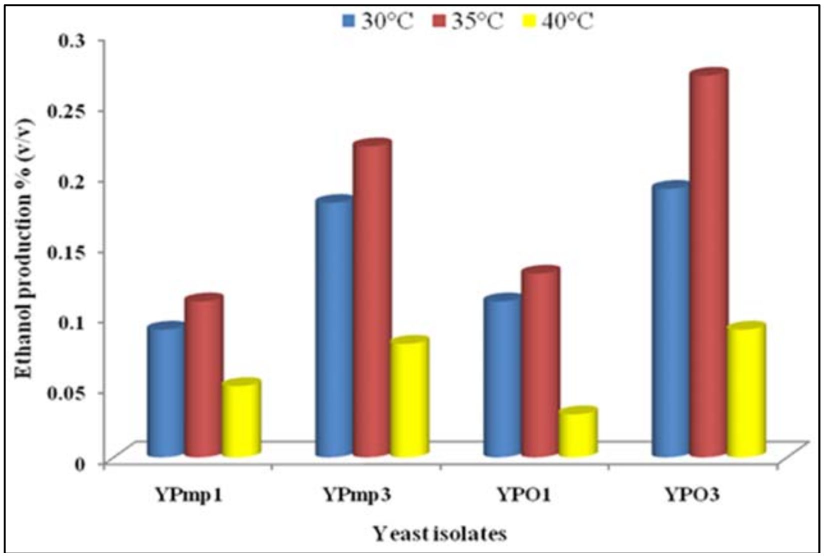

Fig 2: Ethanol production from PPW at different temperature after 72 h

Maximum ethanol was achieved with YPO3 i.e. 0.25% at pH 6.0 whereas. 0.06% was observed at pH 7.0 in PPW after 72 h of fermentation. The ethanol production was decreased with increasing incubation period upto 96 h at all pH. (Fig 3).

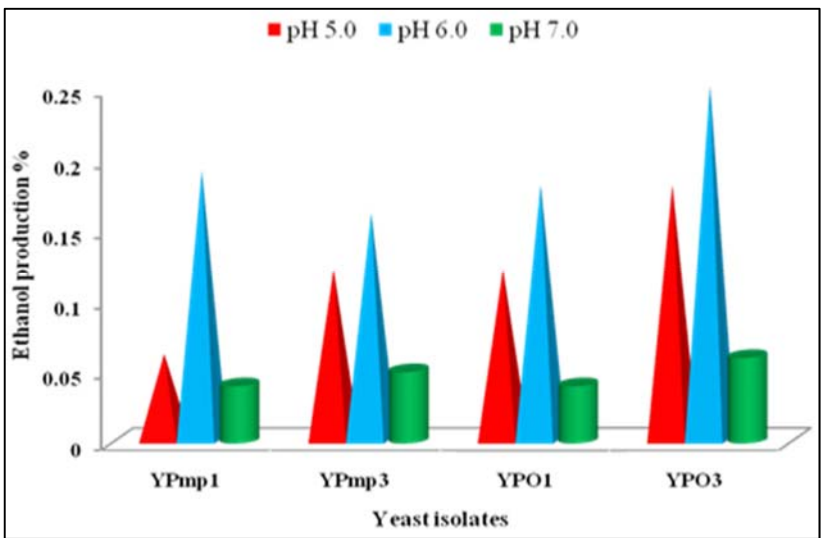

Fig 3: Ethanol production from PPW by yeast isolates at different pH

After optimized temperature and pH different carbon sources such as glucose, maltose and nitrogen sources *viz*., yeast extract and peptone were added to enhance the yeast growth during SHF of PPW. Maximum ethanol in PPW (0.63%) was found in 1.0% glucose whereas 0.26% supplemented with 0.1% yeast extract from PPW at temperature 35°C and pH 6. Duhan *et al.,* (2013) [4] studied bioethanol production from starchy part of tuberous plant (potato) using *S. cerevisiae* MTCC-170. The maximum ethanol concentration (7.95% (v/v)) was obtained with 10% inoculum size at pH 6.0 after 48 h when peptone was used as nitrogen source. Ethanol production from PPW was studied at different incubation period. After 72 h of incubation, the maximum bioethanol was observed in both the substrates at 35°C and pH 6.0 by using four yeast isolates. Optimization of fermentation variables in solid state fermentation and simultaneous saccharification and fermentation methods using co-culture of *Saccharomyces cerevisiae* MTCC 170 and *Aspergillus niger* MTCC 2196 for bioethanol production from waste potatoes was studied by Subhash *et al*., (2016) [14]. The highest yield of bioethanol (5.8%) using co-culture of *Saccharomyces cerevisiae* MTCC 170 and *Aspergillus niger* MTCC 2196 was obtained at incubation temperature of 30°C after 96 h of incubation period. SHF of PPW was carried out at 35°C using two yeast isolates (YPmp3 and YPO3). The maximum ethanol production (2.51%) was observed at temperature 35°C and pH 6.0 after 72 h of fermentation using YPO3 in PPW.

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